

Robust, rapid SEC-MALS titer analysis of AAV particles using 3 μm, 550 Å monodisperse SEC media in bioinert column hardware

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Abstract

Purpose: Demonstrate robust and easy-to-run methods for accurate and precise measurement of AAV titer and aggregates using Thermo Scientific™ SurePac™ Bio 550 SEC MDi™ 3 μm Columns.

Methods: SEC-MALS is used to analyze an in-house AAV5 sample.

Results: Rapid analysis with high precision and increased aggregate resolution compared to columns from another vendor.

Introduction

Adeno-associated viruses (AAVs) are key gene therapy vectors for delivering genetic material to cells for long-term protein production. AAV concentration and aggregate presence affect vector quality, necessitating robust methods for accurate titer and aggregate measurement. Size Exclusion Chromatography (SEC) separates compounds by hydrodynamic volume which correlates with molecular weight. Coupled with Multi-Angle Light Scattering (MALS), SEC-MALS measures AAV titer and aggregates simultaneously. In development and production environments, it is critical to measure these AAV sample characteristics both rapidly and with high fidelity.

This poster presents a case study using SEC-MALS with a 3 μm, 550Å monodisperse media column in 4.6x150 mm bioinert hardware. Robustness was evaluated with multiple analysts, preparation conditions, and media lots. A heavily aggregated AAV5 sample was used for precision and accuracy monitoring, with Bovine Serum Albumin (BSA) as standard. Compared to a 300 mm column, analysis time and sample requirement were halved, with increased aggregate resolution allowing detection of trimer and higher aggregates.

Materials and methods

Sample

AAV5 samples are generated in house in TFS (Alachua, FL, US)

Columns

SurePac™ Bio 550 SEC MDi™ 3 μm Column, 4.6 X 150 mm (P/N 43903-154631)

Conventional SEC column, 5 μm, 500 Å, 4.6 X 300 mm

Data Analysis

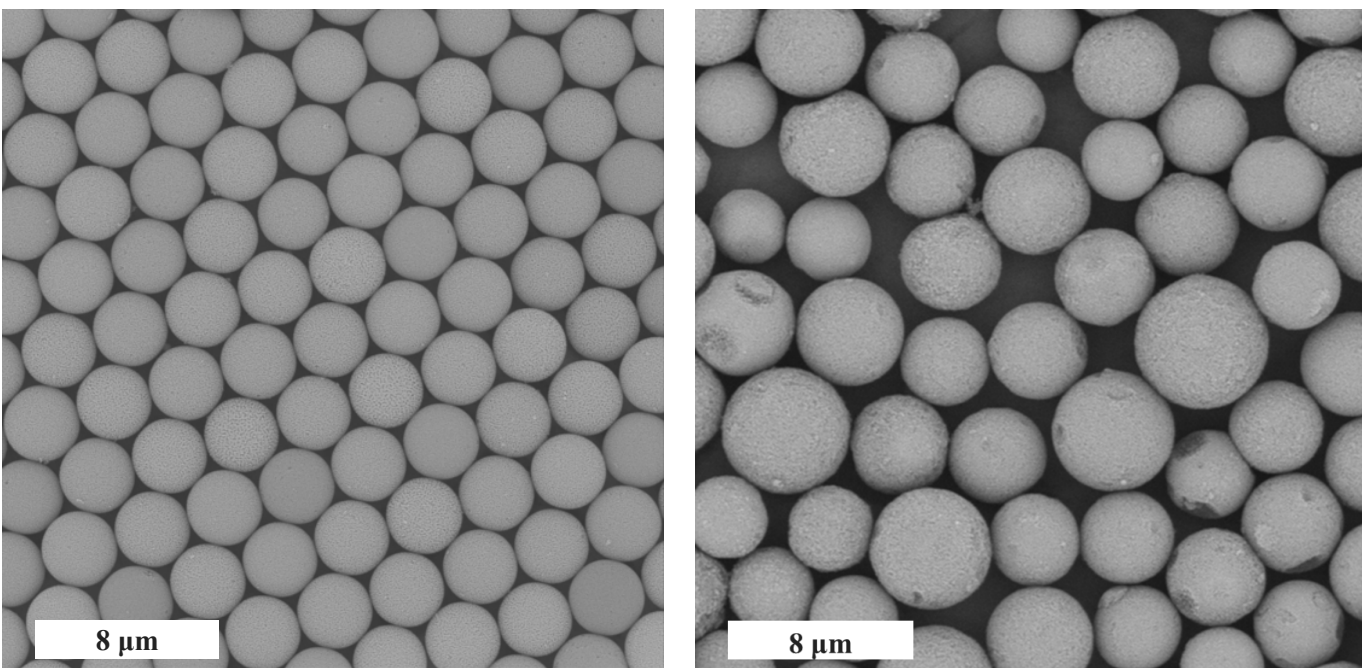
Wyatt Technology ASTRA™ 8.1.2 is used for SEC-MALS data acquisition and analysis.

Results

Monodisperse vs. polydisperse silica particles

The SurePac Bio 550 SEC MDi 3 μm Column monodispersed silica particles are covalently modified with a proprietary diol hydrophilic layer. This proprietary process brings an extremely low level of non-desired interaction sites. Compared to traditional polydisperse particles (right image, Figure 1), the consistent size distribution of the monodisperse particles (left image, Figure 1) not only facilitates precise control over media synthesis and column packing, but also significantly improves column-to-column and lot-to-lot reproducibility. The stationary phase is housed in state-of-the-art hydrophilic-coated stainless-steel hardware. The hydrophilic coating reduces secondary interactions, ensuring optimal performance during the initial injection.

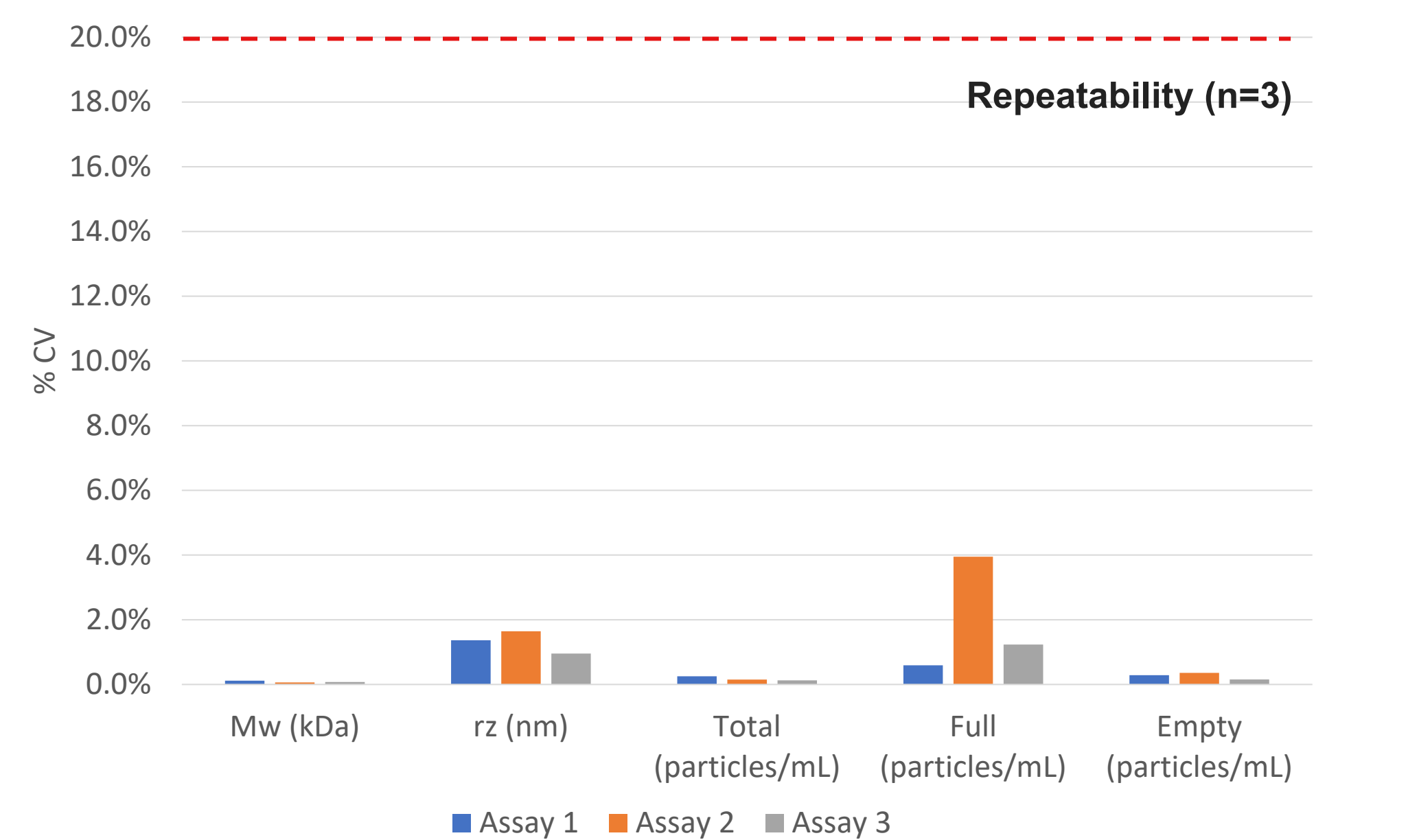
Figure 1. SEM image of 3 μm monodisperse silica particles (left) vs. 3 μm polydisperse particles (right).



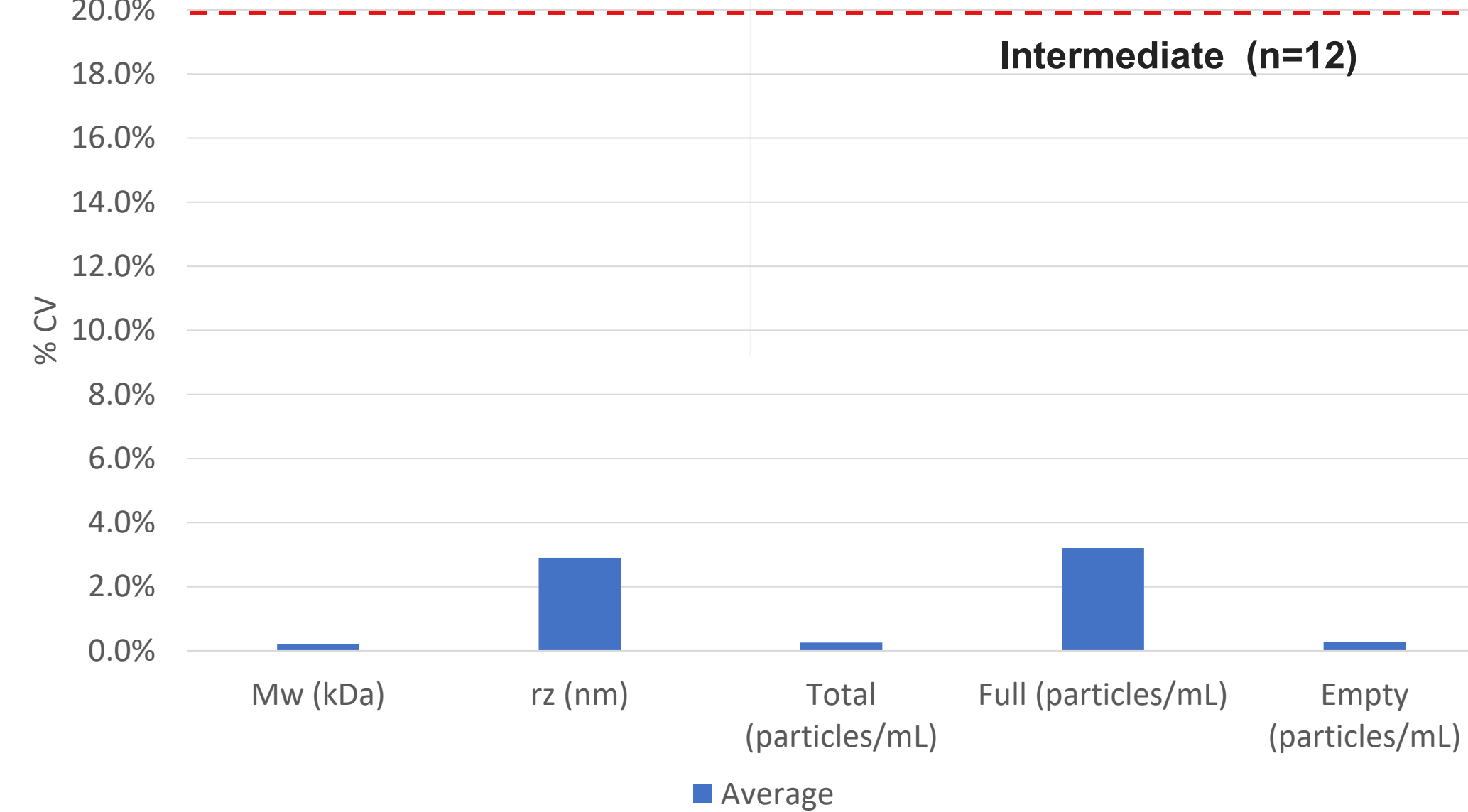
Testing metrics and results

Parameter	Description of Analysis	Acceptance Criteria
PRECISION: Repeatability	• AAV5 tested in at least duplicate (n=2). • % CVs will be calculated.	• % CV ≤ 20% calculated for MW, Size (rz), and Titer (total, full, empty).
PRECISION: Intermediate	• Three runs will be performed over three different days (n= 1, 6&12). • One run performed by Analyst A and two runs by Analyst B.	• % CV ≤ 20% calculated for MW, Size (rz), and Titer (total, full, empty).
Accuracy	• AAV5 material compared with SEC-MALS. • One run performed by Analyst A and Two runs by Analyst B.	• % agreement with respect to MW, Size (rz), and Titer (total, full, empty). • 70-130% % agreement
Column Metrics	• Resolution • Theoretical Plates	• N/A

Figure 2. Repeatability and intermediate results of the AAV5 monomer peak

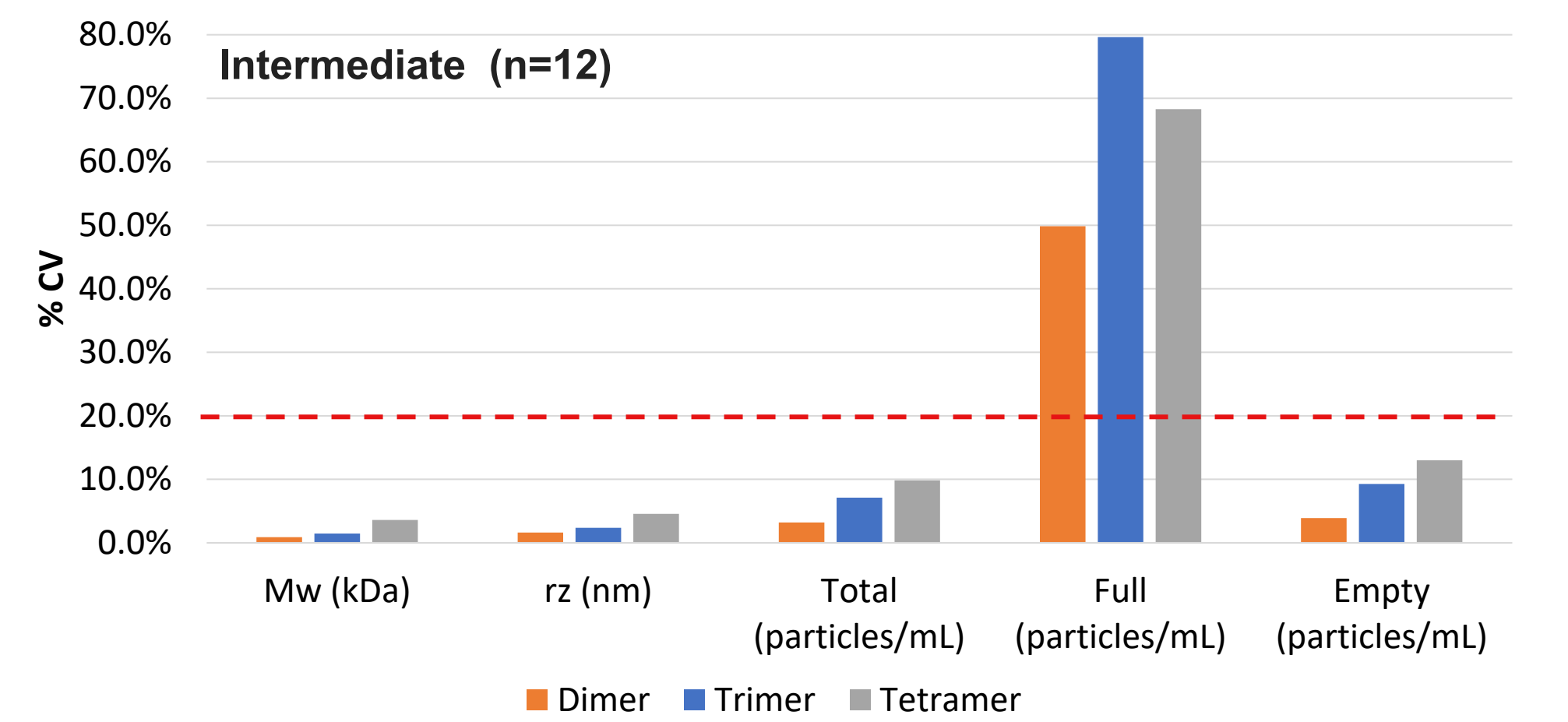
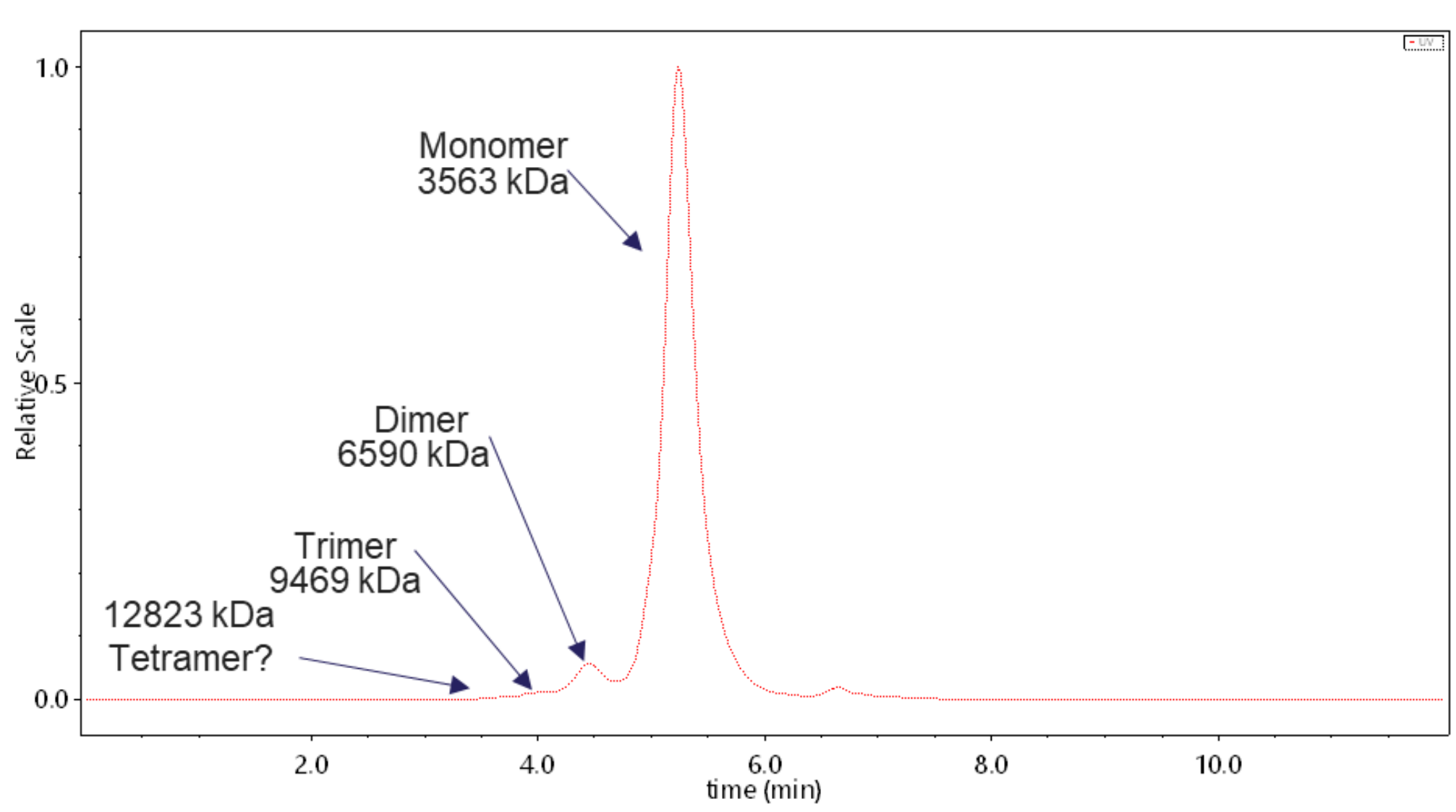


	Assay	Repeatability						PASS/FAIL
		Mw	rz	Total titer	Full titer	Empty titer	Acceptance criteria	PASS
	Assay 1	0.1%	1.4%	0.3%	0.6%	0.3%	%CV ≤ 20%	
	Assay 2	0.1%	1.6%	0.2%	3.9%	0.4%		
	Assay 3	0.1%	1.0%	0.1%	1.2%	0.2%		



Repeatability						PASS/FAIL
Mw	rz	Total titer	Full titer	Empty titer	Acceptance criteria	PASS
0.2%	2.9%	0.3%	3.2%	0.3%	%CV ≤ 20%	

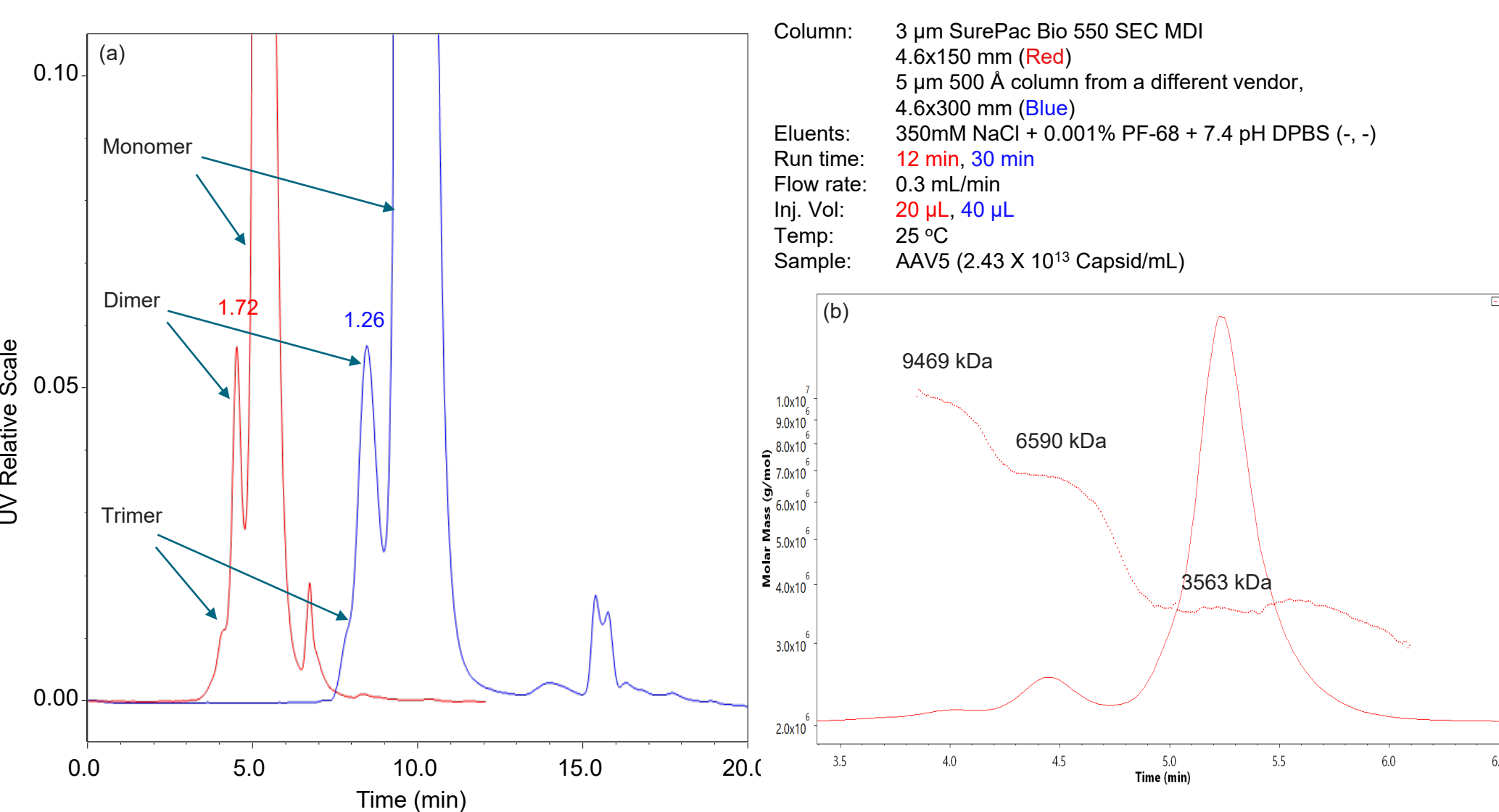
Figure 3. Repeatability and intermediate results of the AAV5 aggregates peaks



	Repeatability						PASS/FAIL
	Mw	rz	Total titer	Full titer	Empty titer	Acceptance criteria	PASS
Dimer	0.9%	1.6%	3.2%	49.8%	3.9%	%CV ≤ 20%	
Trimer	1.5%	2.4%	7.1%	79.6%	9.3%		
Tetramer	3.6%	4.6%	9.8%	68.3%	13.0%		

Figures 2 and 3 demonstrate the excellent repeatability of SEC-MALS data for AAV5 monomer and aggregate peaks, all meeting the acceptance criteria. In Figure 3, the higher %CV for full particles is due to the sample being largely empty, resulting in the full titer being smaller than the LLOQ.

Figure 4. (a) Separation of AAV5 with the SurePac Bio 550 SEC MDi column (Red) and a column from a different vendor (Blue). (b) Molecular mass data using the SurePac Bio 550 SEC MDi column



Analysis time and sample usage are reduced by half or more using the SurePac column compared to the longer conventional column. Even with the reduced separation time, the resolution of the SurePac column is better for the dimer (resolution 1.72 versus 1.26) with improved definition of the trimer shoulder enabling more accurate mass characterization. Plate counts for monomer peaks are more than double with the SurePac column. The enhanced resolution and efficiency offer significant benefits in terms of throughput and mobile phase consumption due to reduced analysis time and reduced sample usage.

Figure 5. Agreement results of the AAV5 monomer peak between the SurePac and the conventional column

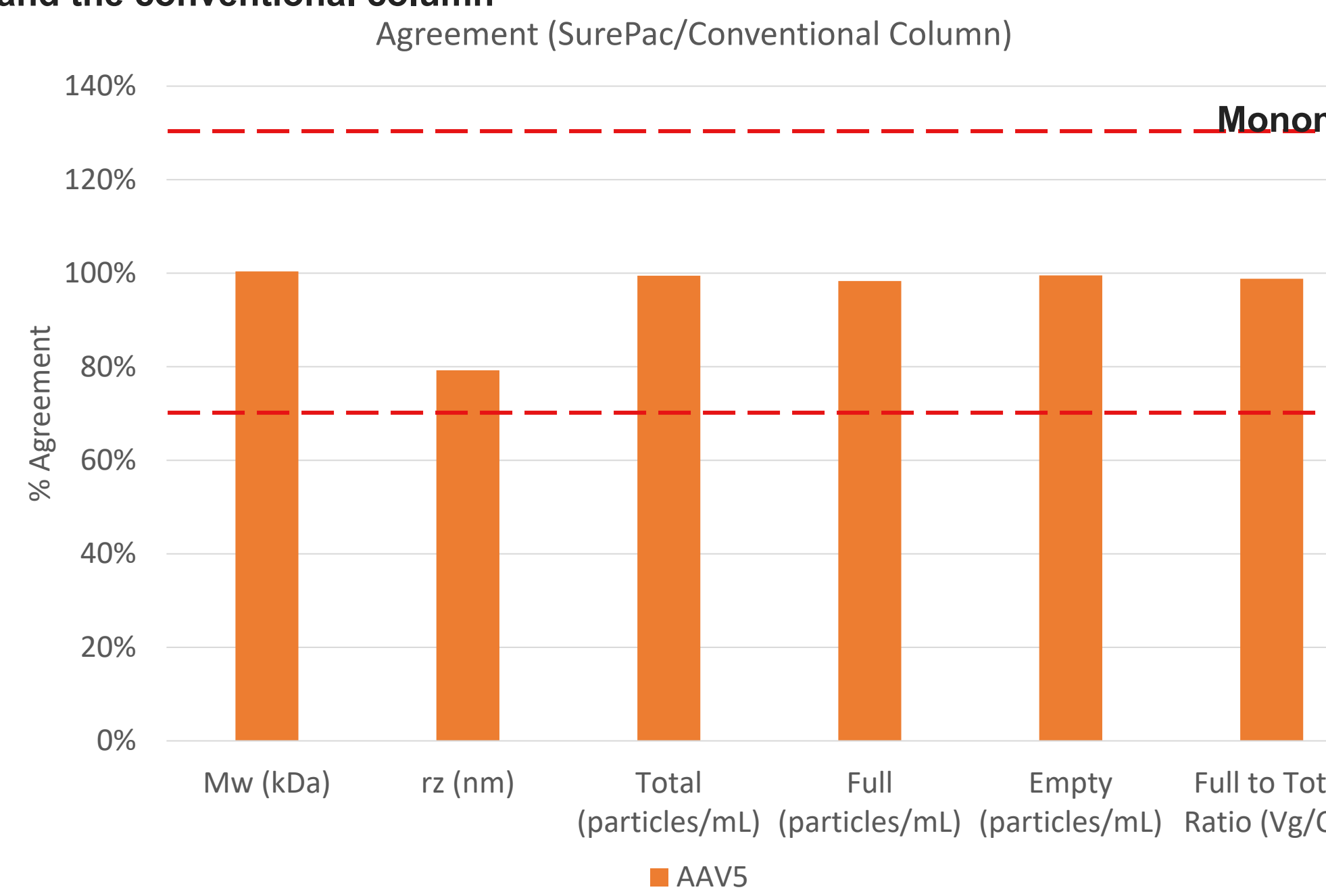
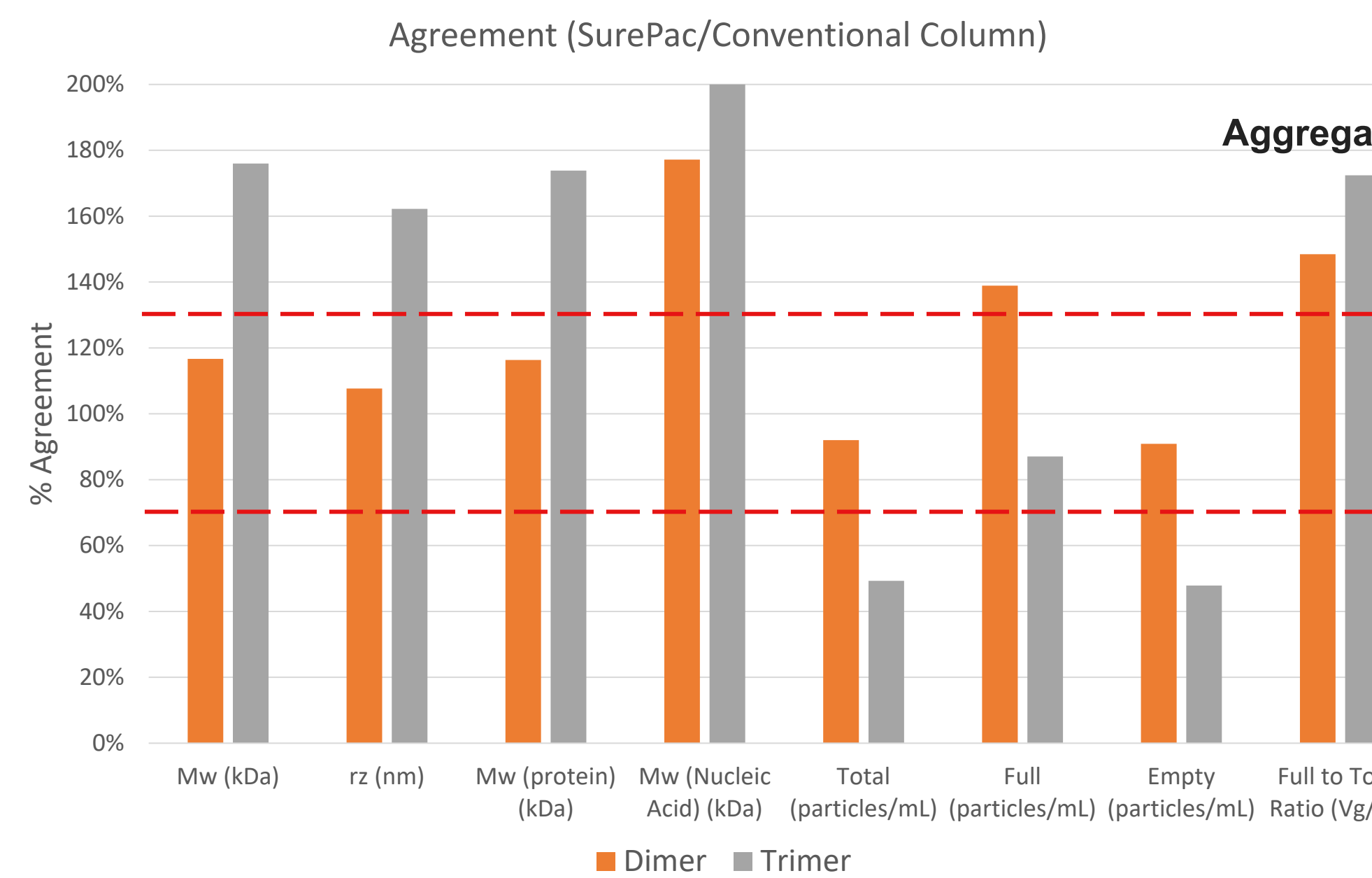


Figure 6. Agreement results of the AAV5 aggregates peaks between the SurePac column and the conventional column



All monomer metrics show good agreement between the two columns. However, the agreement for aggregates is lower, likely due to the conventional column's limited separation compared to the SurePac column.

Conclusions

- The SurePac column reduces analysis time and sample usage by half or more compared to conventional column
- The SurePac column has more than double the plate counts compared to the conventional column
- SEC-MALS is the gold standard for simultaneous measurement of AAV titer, aggregate quantitation (including monomers, dimers, trimers, etc.), and aggregate molecular weight.

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